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Bacteriological Evaluation of Bottled Water from Domestic Brands in Tehran Markets, Iran

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Abstract: Transmission of waterborne disease is a major concern of public health and it is important to know microbial quality of bottled water. This study was conducted about bacteriological evaluation of bottled water from domestic brands in Tehran market. 35 samples of bottled water were collected randomly from seven different brands. The samples were examined about HPC bacteria, coli forms and E.coli according to standard methods for examination of Water. Results showed that the mean and standard deviation of HPC bacteria in examined samples were 3.14×10^2 and 2.07×10^2 CFU/ml, respectively. HPC was ranged from 2.4×10^1 to 9.50×10^2 CFU/ml among bottled water examined. Total coliforms bacteria were detected in 14.28% bottles of mineral water. None of the bottle samples was positive for fecal coliforms and Escherichia coli. The presence of coliforms in bottled water suggests the potential of pathogenic enteric microorganisms and it requires an improved surveillance system in production of bottled water.

Key words: Microbial quality · Water safety · Bottled water · Heterotrophic plate count · Fecal coliforms

INTRODUCTION

Access to safe drinking water is a vital agent for living of human. Water plays a significant role for the sound pathogen that has emerged as a major cause of health [1,2]. Water may transmit some pathogens to human and protection of drinking water in bottles is a method to reduce the pollution occurring by pathogens. Consumption of bottled drinking water has dramatically increased among the community in recent years. Individuals prefer use bottled water in emergency situations or when taste and odor of water is undesirable [3]. In the public opinion, the bottled water is considered completely suitable to drink, but the bottled water sometimes can be contaminated to chemical and biological agents [4]. Most of bottled water in Iran is provided from springs and ground water. Mineral water generally contains inorganic, trace elements and other materials [5]. The city of Tehran has more than 6.7 million people and it has an annual domestic water consumption over 800 million cubic meter (or approximately 90 gallons per capita per day) [6]. There are more than 100 registered manufacturers which provide and pack drinking water in bottles. The consumption of bottled water has been increased particularly in urban population and travelers in Iran during the recent years [7]. Because there is a general belief among consumers that it is safe and free of all impurities, including bacteria [8].

The transmission of waterborne diseases is still a major concern despites considerable efforts and modern technology being utilized for production of safe drinking water [9]. Over the recent years however, concerns have been raised about the microbial quality of drinking water [10-12]. Several studies have documented the detection of coliforms and heterotrophic bacteria in bottled water counts which far exceeded national and international standards set for potable water for human consumption [13]. The overall treatment of source water is dependent in the quality of source water, type of bottled water being manufactured and location [14]. Like any other food product, bottled water must be processed, packaged, shipped and stored in a safe and a sanitary manner and accurately labeled [15]. Mechanical failure, human error or

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deterioration in the quality of source water can lead to failure even in the best treatments systems and disinfection processes [9].

Water quality is often related to the degree of bacterial contamination. The heterotrophic plate count can provide an indication of fast growing bacteria, related to pathogenic types and also an indication of indicator bacteria that develop slowly [16]. In addition to natural contamination, the product can also deteriorate before it reaches the consumer. Therefore, monitoring and determining the microbial quality of bottled water is important to control water borne pathogens. The aim of this study was to evaluate the bacteriological quality of bottled water from domestic brands sold in Tehran markets and their compliance with regulations.

MATERIALS AND METHODS

Sample Collection: 35 samples of bottled water sold in Tehran were obtained from shops and supermarkets. The volume of water bottles was 1.5 liter. These bottles were randomly collected from seven different commercial domestic brands such as Damavand, Damash, Vata, Kouhrang, Siva, Kouhdasht and Nestle by simple sampling. It was selected five samples from each brand with different production dates. All the bottled water samples were transferred directly to the analytical laboratory for bacteriological analysis at suitable conditions.

Sample Examination: The bacteriological analyses were determined according to the standard methods of American Public Heath Association (APHA) for the examination of different types of waters. According this method, the microbiological parameters were determined aerobic and facultative anaerobic heterotrophic bacteria (HPC), total coliforms, fecal coliforms and *E. coli*. The heterotrophic plate count was determined by the pour plate technique as described by the Standard Methods for the Examination of Water and Wastewater [17].

Determination of Heterotrophic Plate Count (HPC): The heterotrophic plate count (HPC) is a procedure and a microbial method for estimating the number of live heterotrophic bacteria in water that uses colony formation on culture media to approximate the levels of heterotrophic flora. Heterotrophs are those microorganisms that use organic compounds for most or all of their carbon requirements. Most bacteria, including many of the bacteria associated with drinking water systems, are heterotrophs. Heterotrophic plate count of all bottled water samples was determined using dilution plate method technique and standard plate count agar medium. Serial dilutions were prepared (using 0.1% peptone water) and 1ml of the sample or dilution was transferred to a sterile, empty petri dish. Plate count agar was melted by heating in boiling water and then allowed to cool in a water bath to 44-46°C. Approximately 15ml of agar medium was poured into the petri dish containing the sample. The sample and agar mixed thoroughly by rotating the plate several times. When the media has solidified, the plates were inverted and incubated at 35°C for 48 to72 hours. Following the appropriate length of incubation, suitable plates from different dilutions were selected and the visible colonies were counted using a colony counter and hand tally. Then, the average colony counts were calculated and expressed as colony forming unit per liter of water (CFU/ml).

Determination of Total Coliforms: Coliforms bacteria were determined by MPN (Most Probable Number) method (5-tubes test) through incubation of sample into tubes of Lactose Broth (LB) at 35°C for 48 h. Then tubes were examined for appearance of gas bubbles within the Durham tubes in Brilliant Green Bile Broth2% (BGBB) at 37°C for 24 to 48 h and the tubes were selected for determination of fecal coliforms which have produced gas. Fecal coliforms are bacteria that are associated with human or animal wastes. They usually live in human or animal intestinal tracts and their presence in drinking water is a strong indication of recent sewage or animal waste contamination [18].

Detection of Escherichia Coli: *E. coli* is a major species of coliform in the fecal coliforms group. It is an indicative of fecal pollution and the possible presence of enteric pathogens. EC broth with Durham tube was used for detection of *E. coli*. A loop of a positive tube was inoculated to EC broth tube and peptone water. Tubes incubated for 24 to 48 hours at 44.5°C. Cultures that produced gas in EC broth tubes and followed by subsequent positive reaction in Indole test were considered as *E. coli* [18].

Statistical Analysis: All statistical analyses were performed using the software SPSS, version 11.5. Analysis of variance ANOVA was employed after logarithmic conversion when necessary to detect significant differences among means. A probability level of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The results of the microbiological analysis carried out on samples of 1.5 L bottles of water from Tehran market appear in Tables 1 and 2. The mean and standard deviation of HPC bacteria in examined samples were 3.14×10^2 and 2.07×10^2 CFU/ml, respectively. HPC was ranged from 2.4×10^1 to 9.50×10^2 CFU/ml among bottled water examined. The brands of Nestle and Kouhrang have the highest and lowest mean amounts of HPC, respectively. 12 from 35 examined bottled water showed with HPC over the maximum level (500 CFU/ml) legally permitted in Iran. On the other hand, only 34.29% samples had bacterial count above 500 CFU/ml. There was a meaningful difference (p-value<0.05) between total mean values of HPC (CFU/ ml) in different samples.

Potable water should be free from any bacteria that might pose a health risk. The presence of HPC more than 500 CFU/ml and coliforms play a main role in the presence of potential pathogens in drinking water. HPC can be as one of the microbiological parameters to be determined in order to control the quality of water for human consumption [19]. In this study brands of Damavand, Damash and Kouhrang had HPC lower than standard level during all sampling periods. According to Iranian regulations, disinfection or sterilization of commercially available mineral water is not permitted. Therefore, generally high HPC can be found a few days after bottling. There are many reports that viable counts can increase to 10^4-10^5 CFU/ml after 1-2 weeks of storage [8, 20-21].

The prevalence of total coliforms (TC), fecal coliforms (FC) and Escherichia coli (E.coli) are shown in Table 3. Coliform bacteria were detected in 5 out of 35 of bottles of drinking mineral water. None of the bottle samples was positive for fecal coliforms and Escherichia coli. The findings showed that 14.28 % of the bottled water sampled in the current study contains coliform organisms and they failed to meet the World Health Organization standard for coliforms in bottled water [22]. The presence of coliforms in bottled water suggests the potential presence of pathogenic enteric microorganisms and it requires an improved surveillance system for the bottled water industry. In drinking water from bottled water, the coliform test can be used as an indicator of treatment efficiency and of the integrity of the production system.

Coliform bacteria are the only microbiological contamination to be regulated by law in bottled water. For bottled water, the Iranian directives state that coliforms from bottled water source must not be detectable in any 100ml sample. The total coliform of bottled water according to EPA, Iranian drinking water standards and WHO guidelines is zero [23-25]. According to this study, total coliforms in 100 ml water were also zero among the brands of Damavand, Vata, Kouhrang and Siva. It was more than zero in brands of Damash, Kouhdasht and Nestle (Table 3). There was meaningful difference (p-value<0.05) between total mean values of coliforms /100 ml in different sample and it shows that the quality of these bottled waters is somewhat unhealthy for public consumption. Also, E.coli was assayed for positive samples but it was not detected in any of the samples in the present study. Although none of the brands were isolated *E. coli* but it may exit in other brands that have not been examined, because *E. coli* has the potential to multiply and then survive for over 40 days in artificially inoculated bottled water [14-26]. The presence of *E. coli* in water is nearly always associated with recent fecal pollution and it is the preferred indicator organism for this purpose [17]. In the present study, neither *E. coli* nor Fecal coliforms were found in any of the examined bottled water samples. It suggests the absence of fecal contamination at these samples.

Coliforms bacteria and HPC both accepted as microbial contamination indicators by WHO, EPA and other related different international organizations. The presence of coliform bacteria can indicate diseases causing pathogens in water. Therefore, the consumption of contaminated bottled waters should be limited [27].

Table 1: Level of Heterotrophic plate counts (HPC) (CFU/ ml) in domestic brands of bottled water in Tehran Markets

Brand name	No. samples tested	Range	Mean ±SD
Damavand	5	6.9×10^1 - 4.52×10^2	$2.39\times10^2\pm 1.58\times10^2$
Damash	5	1.93×10^2- 7.15×10^2	$4.6\times10^2 \pm 2.37\times10^2$
Vata	5	6.7×10^1- 4.20×10^2	$2.37\times10^2\pm 1.66\times10^2$
Kouhrang	5	2.4×10^1- 3.22×10^2	$1.56\times10^2 \pm1.45\times10^2$
Siva	5	8.6×10^1 - 5.2×10^2	$3.06\times10^2 \pm1.54\times10^2$
Kouhdasht	5	3.4×10^1- 9.50×10^2	$2.6\times10^2\pm 3.36\times10^2$
Nestle	5	2.33×10^2- 8.44×10^2	$5.42\times10^2\pm2.58\times10^2$
Overall	35	2.4×10^1 -9.50×10^2	$3.14\times10^2 \pm2.07\times10^2$

Table 2: Distribution of Heterotrophic plate counts in bottled water from commercial brands in Tehran markets

Indicator bacteria	Number (%) of sample positive (n=35)
HPC (CFU/ml) <1	0 (0.0)
1-500	23 (65.71)
>500	12 (34.29)
Range	65-950
Mean	314

Table 3: Prevalence of total coliforms, fecal coliforms and *E. coli* in sampled bottled water according to brands of purchase

		No. (%) of samples positive		
Brand name	No. samples tested	[1]TC	[2]FC	[3]*E. coli*
Damavand	5	0 (0.0)	0 (0.0)	0 (0.0)
Damash	5	2 (40)	0 (0.0)	0 (0.0)
Vata	5	0 (0.0)	0 (0.0)	0 (0.0)
Kouhrang	5	0 (0.0)	0 (0.0)	0 (0.0)
Siva	5	0 (0.0)	0 (0.0)	0 (0.0)
Kouhdasht	5	1 (20)	0 (0.0)	0 (0.0)
Nestle	5	2 (40)	0 (0.0)	0 (0.0)
Overall	35	5 (14.28)	0 (0.0)	0 (0.0)

[1]Total coliforms, [2]Fecal coliforms and [3]Escherichia coli

In present study, the brands of Damash, Kouhdasht and Nestle are positive from coliforms bacteria and they are not suitable for consumption. Unfortunately there is no regular inspection and monitoring to control bottled waters in Iran. According to EPA and Iranian drinking water standards and also WHO guidelines, HPC in bottled waters must be less than 500 colonies per milliliter [23-25]. HPC can indicate the microbial quality of water like MPN for coliforms bacteria. If HPC is more than standard in bottled water it can be undesirable to drink such waters, because it may cause disease in consumer [25].

In a study, Abayasekara *et al.* [28] surveyed 34 different brands of bottled water in Seri Lanka, found that 5 out of the 34 brands (15 %), tested were positive for fecal coliforms, indicating concern over the microbiological quality of bottled water.

A study conducted by Ehlers *et al.* [29] in South Africa showed that eight local and two brands of bottled water were free of total and fecal coliforms of bacteria. In a research, Abed and Alwakeel (2007) reported that there is contamination with some bacteria such as Bacillus cereus and Pseudomonas in two of the 30 (6.7%) bottled samples [30]. It is important to identify of contaminated bottled water and to prevent the consumption of these samples.

CONCLUSIONS

Based on the recommended zero tolerance for coliforms in drinking water by EPA and WHO and 14.28 % of water bottled from domestic commercial brands in Tehran markets could be considered unsuitable for human consumption. Consumers of contaminated water such as immuno-compromised persons, elderly or infants are sensitive to pathogens and they should make aware from this problem. In addition, utilization of bottled water for reconstituting foods of infants and patient persons may threat the health of these groups. So, public awareness and education is important about the quality and health risks of bottled water in Tehran market. It is necessary to provide measure to enforce microbial standards and laws for bottled water sold in Tehran market by government. Application of good manufacturing practices, strict process control and personnel hygiene should be maintained at processing facility. Implementation of HACCP (Hazard Analysis Critical Control Points) for the microbial quality of bottled water in all processing from raw water to final products, to control contamination during bottling and prohibit microbial growth during storage and distribution can be terminated to receive safe water.

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